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Leaf Flavonoids as Chemotaxonomic Characters in Genus *Sida* L. (*Bevila*)

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ABSTRACT. *The flavonoid distribution pattern of five species of genus Sida was studied to identify the species since the existing classification was mainly based on morphological and anatomical characters. Four main flavonoids and one minor flavonoid were isolated by preparatory paper chromatography. Trace amounts of six flavonoids were observed in two-dimensional paper chromatography. The major flavonoid compounds in S. acuta, S. rhombifolia, S. alnifolia, and S. cordifolia were glycosylated and methylated flavonols where as S. humilis had minor flavonoids. There are remarkable variations in the substitution patterns of these flavonol compounds. The presence of quercetin 3-methyl ether and the compound C is a common character to S. rhombifolia and S. cordifolia, and hence these two species may be considered as related taxa in the genus Sida. Kaempferol glycosides were the major flavonoids in the S. alnifolia in contrast to the S. rhombifolia, which was characterised by the presence of methylated quercetin. This is in agreement with the recent revision of the genus in which they are considered as different entities. The habit of S. humilis, prostrate trailing, is different from other species, which are erect herbs. This dissimilarity of morphology agreed with its characteristic flavonoid pattern of possessing minor flavonoids.*

INTRODUCTION

The genus *Sida* L. (family: Malvaceae) comprises 120 cosmopolitan herbaceous species, of which seven species are found in Sri Lanka. Trimen (1974) reported six species and one variety of *Sida rhombifolia* in his Flora of Ceylon. Dassanayake and Fossberg (1998) promoted this variety. *S. rhombifolia* var β retusa to a species (*S. alnifolia*) in a recent revision of the genus. These species are considered as weeds in most countries, whereas in Asian region these are widely used in many indigenous medicinal preparations (Jayaweera, 1982). Description of different species is mainly based on morphological and anatomical characters, which are highly variable within species making identification ambiguous. For instance, in *S. rhombifolia* and close relatives, the shape of the leaves varies from small, linear to lanceolate, large and round; flower colour varies from yellow to orange. Therefore, use of many characters with less variability is suggested to construct reliable descriptions for the species. Chemical approach is considered to provide additional as well as reliable support in modern taxonomy (Jones and Luchsinger, 1986). As flavonoids have been proved to be a good marker compound in solving taxonomic problems over the years, the flavonoid profiles of *Sida* were used in the present study. Additionally, other biochemical markers and/or morphological data could provide useful characters for defining species in the genus *Sida*.

The major objective of this study was to investigate the possibility of using flavonoids as taxonomic characters to identify the various taxonomic levels in the genus *Sida*, and further to distinguish species, and to characterize sections or subgenera.

MATERIALS AND METHODS

Plant material

Leaves of five species of the genus *Sida* were collected from natural habitats for this study. The other two species were not found. Herbarium specimens were prepared and the authenticities were checked with those at Royal Botanic Garden, Peradeniya. Voucher specimens were submitted to the departmental herbarium, University of Kelaniya.

Extraction

For extraction, 200 mg of dried, ground plant material was pulverized in a test tube and 5 ml of 80% aqueous methanol was added. The test tubes were heated in a water bath at 90°C for two minutes. The test tubes were then cooled, and left in the 80% methanol for 20-24 h to extract at room temperature. Subsequently, the extracts were filtered and evaporated in *vacuo* at 40°C. For the isolation of compounds the same method was used with 20 g of dried leaf material (Harborne, 1998; Markham, 1982).

Two-dimensional paper chromatography (2-D PC)

Each dried extract to be analysed was dissolved in 1 ml of 80% methanol. Then, 8-10 drops from each extract were applied in the corner of quarter sheets of Whatman No. 1 chromatography paper. The chromatograms were run by descent in BAW (n-butanol : acetic acid : distilled water - 4:1:5 upper layer), first direction, and in 15% aqueous acetic acid, second direction. After drying, the chromatograms were viewed in 366 nm under UV light, and then again after fuming with ammonia vapour. The colours of spots were recorded and R_f values were calculated (Harborne, 1998).

Preparative paper chromatography (PPC)

Each extract was applied as a streak on Whatman No. 3 chromatography papers, and the papers were run by descent in BAW. After drying, the chromatograms were viewed under UV. Flavonoid glycoside bands with different colours were cut into pieces after marking with a pencil. Then cut pieces were eluted in 80% methanol. The purification process was continued using 15% acetic acid and distilled water until a single band appeared (Markham, 1982).

Acid hydrolysis

Purified flavonoid glycosides were hydrolysed with 2M HCl for 30-40 min at 100°C and extracted with ethyl acetate. The extracts were evaporated and dissolved with few drops of 95% ethanol and run in Whatman No. 1 papers in solvents, BAW and Forestal (conc. Hcl : acetic acid : distilled water - 3:30:10) with standard markers. Aqueous layers of the extracts were concentrated in vacuo at 40°C and were spotted in Whatman No. 1 chromatography papers and along with a mixture of standard sugars, were run in solvents, phenol, BAW for the separation of the sugars. Dried chromatograms were dipped in aniline hydrogen phthalate and heated in an oven at 100-120°C for few minutes until spots appeared (Markham, 1982).

UV-Visible absorption spectroscopy

The spectral maxima of the purified flavonoid compounds were obtained for methanol using UV-Visible spectrophotometer. Substitution patterns of the flavonoid compounds were determined by adding shift reagents: CH_3COONa, H_3BO_3 and NaOH.

RESULTS AND DISCUSSION

Identification of the flavonoid compounds

The preliminary study (2-D PC) revealed the presence of flavonoids in crude extracts of five species studied (Table 1). Based on this finding, four main flavonoid compounds (referred to as compound 1-4) and one minor flavonoid compound were isolated by PPC. Trace amounts of six flavonoid compounds (referred to as compound A-F) were observed in 2-D PC (Table 1, 2, 3 and 4).

Compound 1 was identified as a flavonol compound with C- and O-glycosylation. According to the spectral maxima and R_f values aglycone was identified as a flavonol. The presence of C-glycosides was determined by its extractability in amyl alcohol, but not in ethyl acetate (Harborne, 1998). The separation of the hydrolysed compound into two spots in forestal chromatogram provided further evidence for the presence of C-glycosides. Arabinose was found as a sugar moiety, hence the O-glycosylation was confirmed. The compound 2 was identified as kaempferol with two O-glycosides. Aglycone was confirmed as kaempferol and two sugars were identified as arabinose and glucose by their spectral properties and R_f values. Compound 3 was identified as quercetin-3-methyl ether by its chromatographic and spectral properties. Identity was confirmed by comparing its spectrum with reference spectrum of Mabry *et al.* (1970). Although Harborne (1967) reported that the methylated flavonoids were rare in angiosperms, quercetin 3-methyl ether was found in *S. rhombifolia* and *S. cordifolia* in this study. Aglycone of the compound 4 was identified as kaempferol, and further studies need to be carried out to identify its glycosylation pattern.

Table 1. Flavonoid glycosides in the leaves of genus *Sida* (2-D PC).

Species	Colour (UV/UV+NH_3)	R_f values		Compound
		BAW	15% acetic acid	
S. acuta	BY/BY	88	48	1
	D/Yg	75	60	2
	Flb/LFlb	84	74	C
	Purple/Yg	40	64	A
S. rhombifolia	D/Y	62	56	3
	Y/Y	58	38	B
	Flb/LFlb	70	75	C
S. alnifolia	D/Y	60	43	4
	Y/Y	5	37	B
S. cordifolia	D/Y	64	56	3
	Y/Y	46	64	D
	Flb/LFlb	70	77	C
S. humilis	Purple/Y	23	55	minor flavonoid
	Flb/Yg	41	87	E
	Blue/Yg	40	89	F

BY - Bright Yellow; D - Dark mauve; Flb - Fluorescent blue;
LFlb - Light Fluorescent blue; Y - Yellow; Yg - Yellowish green

However, R_f values were in support of O-glycoside of kaempferol. The compound 2 and the compound 4 were both kaempferol glycosides and differed only by their spectral maxima in band I, which suggests the differences in substitution pattern. The chromatographic characters of the compound isolated from *S. humilis* were such that it belonged to minor flavonoid group (Markham, 1982). Further investigation is needed for structure elucidation of this compound.

Table 2. Chromatographic properties of the aglycones.

Compound	Colour (UV/UV+NH_3)	R_f values		Sugar moiety
		Forestal	BAW	
1	BY/BY	90	84, 54	Arabinose
2	Y/Y	53	83	Arabinase
				Glucose
3	Y/Y	42	64	Not detected
4	Y/Y	53	86	Not analysed
Minor flavonoid	Y/Y	78	16	Not analysed

BY and Y refer as Bright Yellow and Yellow respectively.

Table 3. UV-Vis spectral properties of flavonoid compounds.

Compound	Flavonoid type	UV-Vis spectral values			
		Methanol	NaOH	CH_3COONa	H_3BO_3
1	C,O-glycosylated	245, 275, 282,	+	-	-
	Flavonol	355 sh, 370	+	+	-
2	Kaempferol -diglycoside	266, 298 sh, 350			
	Kaempferol (aglycone)	244 sh, 265, 315 sh, 358	+ (D)	-	-
3	Quercitin 3-methyl ether	257, 269 sh, 294 sh, 358	+	+	+
4	Kaempferol-glycoside	265, 300 sh, 343	+	+	-
Minor flavonoid		232 sh, 300 sh, 324	+	-	+

+ - bathochromic shift D - decomposition present - no shift

Comparison of flavonoid profiles among different species

The major flavonoid compounds in *S. acuta*, *S. rhombofolia*, *S. alnifolia*, and *S. cordifolia* were glycosylated and methylated flavonols with the exception of *S. humilis* which had minor flavonoids. This is a deviation from the view that the herbaceous plants have more flavones than flavonols and also considered as an advanced character (Harborne, 1967). There are remarkable variations in the substitution patterns of these flavonol compounds, for instance the compound 3, common to *S. rhombofolia* and *S. cordifolia* was a methylated flavonol and C-glycosylated flavonol was found in *S. acuta*.

Out of the five species studied, *S. acuta* was found to be rich in flavonoids and compound 1 was found in relatively large amounts (Table 4). Furthermore, both O-glycosylation and C-glycosylation of flavonols were recorded in *S. acuta*. Therefore, these compounds can be used as taxonomic characters of *S. acuta*.

The presence of quercetin - 3 methyl ether and compound C, is a common character to *S. rhombifolia* and *S. cordifolia*, and hence these two species can be considered as related taxa in the genus *Sida*. Kaempferol glycoside was the major compound found in the *S. alnifolia* in contrast to the *S. rhombifolia*, which was characterized by the presence of methylated quercetin compound. The present study revealed that these two taxa were different in their flavonoid profile and it is in agreement with the recent revision of the genus, which was based on anatomical and morphological characters. *S. humilis* showed a significant difference from other species in its flavonoid type, by possessing minor flavonoids. *S. humilis* is a prostrate trailing herb with long branches, rooting at the nodes, lacking woody parts and this habit is different from other species, which are erect herbs. This dissimilarity of morphology agreed with its characteristic flavonoid pattern that was different from other species. The relationship of the flavonoid composition among the species was presented in a dendogram (Fig. 1).

Table 4. Distribution of flavonoid compounds in the genus *Sida*.

Species	Compound*										
	1	2	3	4	mf	A	B	C	D	E	F
S. acuta	+ +	+	-	-	-	±	-	±	-	-	-
S. alnifolia	-	-	-	+	-	-	±	-	-	-	-
S. rhombifolia	-	-	+	-	-	-	±	±	-	-	-
S. cordifolia	-	-	+	-	-	-	-	±	±	-	-
S. humilis	-	-	-	-	+	-	-	-	-	±	±

mf - minor flavonoid; ++ - high amounts; + - low amounts; ± - trace amounts; - not detected
*Compound 1, 2, 3, 4, A, B, C, D, E, F and minor flavonoid are described in Table 1.

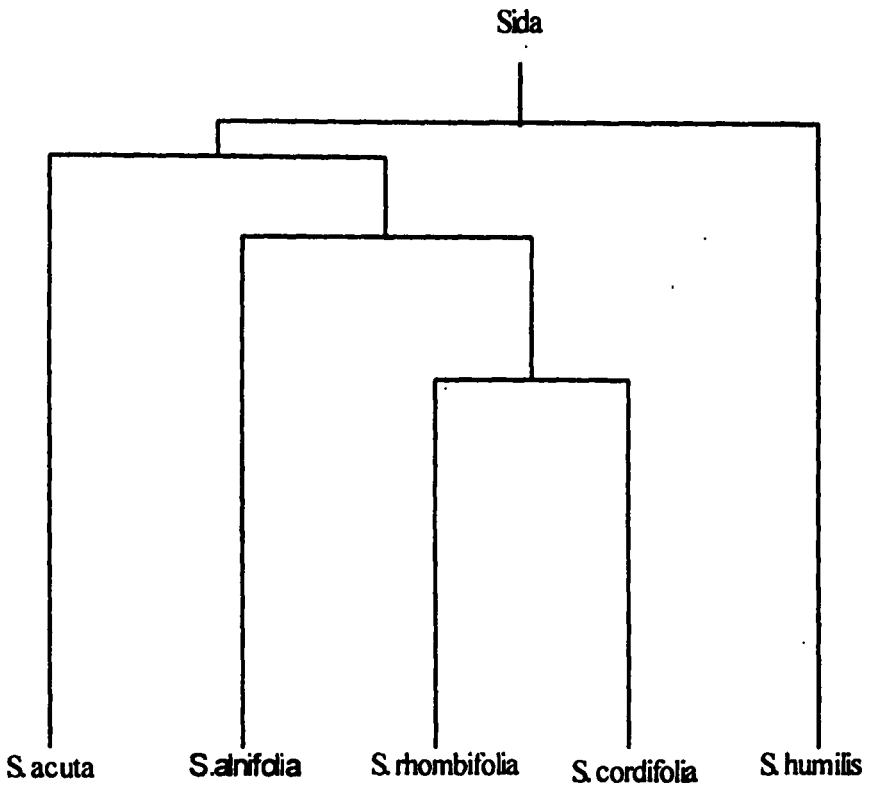

Fig. 1. Dendogram showing the relationships of the flavonoid composition among the *Sida* species.

CONCLUSIONS

Flavonoid distribution pattern of the genus *Sida* is in agreement with the existing classification system and supports the recent revision. Specially, flavonoid composition has a relationship with the taxonomic position of the *S. rhombifolia* and *S. alnifolia* and in favour of treating them as separate entities. Flavonoids can provide important taxonomic characters for the species *S. humilis* and *S. acuta*, which can be characterized by the presence of minor flavonoids and C-glycosides, respectively. Therefore, flavonoids can be considered as taxonomic characters for the genus *Sida* along with other biochemical and structural data. This information would be useful in a numerical taxonomic treatment in the future.

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